Clinical translation of stem cell therapy in traumatic brain injury: the potential of encapsulated mesenchymal cell biodelivery of glucagon-like peptide-1

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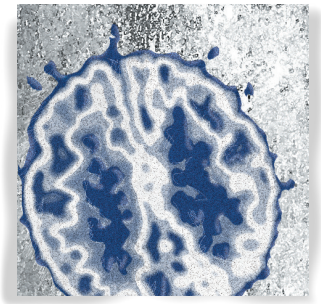

Traumatic brain injury remains a major cause of death and disability; it is estimated that annually 10 million people are affected. Preclinical studies have shown the potential therapeutic value of stem cell therapies. Neuroprotective as well as regenerative properties of stem cells have been suggested to be the mechanism of action in preclinical studies. However, up to now stem cell therapy has not been studied extensively in clinical trials. This article summarizes the current experimental evidence and points out hurdles for clinical application. Focusing on a cell therapy in the acute stage of head injury, the potential of encapsulated cell biodelivery as a novel cell-therapeutic approach will also be discussed.

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According to the World Health Organization, traumatic brain injury (TBI) will surpass many diseases as the major cause of death and disability by the year 2020. It is estimated that 10 million people are affected annually by TBI,[1] with the highest incidence among persons 15 to 24 years of age and 75 years and older.[2] Since TBI may result in lifelong impairment of an individual's physical, cognitive, and psychosocial functioning, and given the absence of a cure, TBI is a disorder of major public health significance.

Stem cell therapies hold promise for the treatment of various human diseases, including TBI. However, the lack of basic knowledge concerning basic stem cell survival, migration, differentiation, and integration in a real-time manner when transplanted into damaged central nervous system (CNS) remains a problem in attempts to design stem cell therapies for CNS diseases. Several types of stem cells have been investigated for the treatment of diseases of the CNS. Embryonic stem cells (ESCs) are pluripotent cells that have the capability to differentiate into nearly all cell types, including neuronal and glial fate cells.[3] However, the safety of transplanting ESCs in humans has not been established so far, one concern being the controversial formation of teratomas following ESC-derived neural cell engraftment.[4] Neural stem cells (NSCs) are multipotent cells with the potential to differentiate into neurons, oligodendrocytes, and astrocytes and can be efficiently propagated in vitro.[5,6] However, many critical challenges remain using NSCs for clinical applications, including the need for pure populations of differentiated cells, inefficient tracking systems, and moderate cell survival after transplantation.[6,7] A third option is the use of mesenchymal stem cells

Selected abbreviations and acronyms

ESC *embryonic stem cell*
NSC *neural stem cell*
GLP *glucagon-like peptide*
MSC *mesenchymal stem cell*
hMSC *human bone marrow-derived mesenchymal stem cells*
CCI *controlled cortical impact*
MAP *microtubule-associated protein*
GFAP *glial fibrillary acidic protein*

(MSCs), which have been reported to elicit neuroprotective and regenerative effects following cerebral ischemia and TBI.[8,9] The cells may be administered intravenously, but direct intracerebral administration has been suggested to be potentially more effective.[10] It has been shown that MSCs act mainly through the release of neurotrophic and immunomodulatory peptides, as opposed to through cell replacement or direct cell-to-cell contact.[11,12] Given this, exogenous cells may well be a source of trophic support, promoting endogenous repair such as neurogenesis, angiogenesis, and synaptogenesis.[13]

Mechanisms of action of stem cell therapy in CNS injury

The neuroprotective effect of stem cells for the treatment of CNS injury has been shown in several preclinical studies. However, the exact mechanism remains controversial. Potential mechanisms currently under investigation include engraftment and transdifferentiation, modulation of the inflammatory milieu, and modulation of the systemic immunologic/inflammatory response.

Lundberg et al[14] administered human mesenchymal stem cells in the ipsilateral internal carotid artery of rats which had been subjected to experimental TBI. Intra-arterial transplantion of mesenchymal stem cells resulted in CNS engraftment without thromboembolic ischemia. Kuh et al[15] implanted human umbilical cord blood-derived progenitor cells (HUCBCs) into the injury site after spinal cord contusion in a rodent model. The transplanted HUCBCs were differentiated into various neural cells, which were confirmed by double immunofluorescence staining of bromodeoxyuridine (BrdU) and glial fibrillary acidic protein (GFAP) and microtubule-associated protein-2 (MAP-2) staining. Locomotor testing showed functional improvement for all time points tested up to 8 weeks after spinal cord injury. Salazar et al[16] transplanted human neural stem cells into immunodeficient NOD-scid mice 30 days post spinal cord contusion injury. The transplanted mice demonstrated significantly improved locomotor recovery compared with vehicle controls using open field locomotor testing and CatWalk gait analysis. The transplanted neural stem cells exhibited long-term engraftment, migration, limited proliferation, and differentiation predominantly to oligodendrocytes and neurons. Also, differentiated NSCs integrated with the host as was demonstrated by colocalization of human cytoplasm with discrete staining for the paranodal marker contactin-associated protein.

Dramatic cerebral responses following TBI comprise inflammation, cell death, and modulation of trophic factor release. These cerebral modulations might be influenced by stem cells. Walker et al[17] directly implanted MSCs into the brains of rats which had been subjected to TBI. Brain supernatant analysis showed an increase in interleukin (IL)-6, which has both direct and indirect neurotrophic effects on neurons.[18] Glazova et al[19] implanted neuronal phenotype ES cells in mice after experimentally induced spinal cord injury. Transplantation of the ES cells activated both brain-derived neurotrophic factor IL-6 signaling pathways in the host tissue, leading to activation of cAMP/PKA, phosporylation of cofilin and synapsin I, and promoting regenerative growth and neuronal survival. Given the results of these preclinical studies, modulation of the proinflammatory environment could afford neuroprotection.

Models of TBI invariably show activation of microglial cells, although it is unclear whether such activation promotes neuronal survival, or exacerbates neuronal damage.[20] Also, adaptive immune responses play a role. For example CD4+ T cells are observed in the substantia nigra in TBI patients,[21] and in a model of spinal cord injury, T cells isolated from diseased animals induced transient hind limb paralysis and spinal cord inflammation when injected into naïve recipients.[22] B cells in this model were also pathogenic. Although innate responses are considered protective, there is a delicate balance between the innate immune system and the adaptive immune system in mediating either pathogenic or repair processes under these conditions.[22] Walker et al[23] were able to show that the intravenous injection of multipotent adult progenitor cells after experimental TBI in rodents preserved splenic mass and increased the num-

ber and proliferative rate of CD4+ T cells as well as the production of IL-4 and IL-10 in stimulated splenocytes. Hence, the colocalization of transplanted MAPC and resident CD4+ splenocytes seems to be associated with a global increase in IL-4 and IL-10 production and stabilization of the cerebral microvasculature tight junction proteins. Nemeth et al[24] administered bone marrow stromal cells to mice before or shortly after inducing sepsis by cecal ligation and puncture, and found monocytes and/or macrophages from septic lungs made more IL-10 when prepared from mice treated with bone mesenchymal stem cells (BMSCs) versus untreated mice, leading to reduced mortality and improved organ function.

Clinical translation of stem cell therapy in TBI

Step 1: Deciding on an approach

Despite the promising preclinical results described above, there are problems to consider when trying to translate these studies into a clinical setting. First and foremost, the importance of engraftment and transdifferentiation remains controversial. Intravenous infusion of MSCs in rats which had been subjected to TBI failed to result in significant acute or prolonged cerebral engraftment of cells or to modify the recovery of motor or cognitive function.[25] Also, the transplantation of neuronal stem cells into the ipsilateral or contralateral corpus callosum of rats at 48 hours after severe experimental TBI failed to lead to proliferation of the implanted cells, regardless of the site of implantation.[26] Cao et al[27] found pluripotent stem cells engrafted into the normal or lesioned adult rat spinal cord to be restricted to a glial lineage. Zheng et al[28] implanted neural stem cells derived from Wistar rats into traumatized Sprague-Dawley rats and studied the local lymphocyte infiltration. The histological examination and immunohistochemistry revealed significant lymphocyte infiltration in the contusion, suggesting that immunosuppressive treatment is necessary following NSC transplantation. Considering these problems, this pathway may not be feasible for a clinical translation at this point in time.

Therefore, "encapsulated cell biodelivery" has been put forward as a novel clinical strategy for cell therapy in the CNS. Encapsulation was originally introduced to assist in allowing allogenic or xenogenic cell transplantation. It appears that semipermeable hollow fibers,[29] as well as spherical polymeric microcapsules,[30] protect cells transplanted into the brain from the immunological graft-versus-host response. As the capsules permit the free passage of nutrients, oxygen, and, indeed, smaller molecules, the cells are maintained within the capsules, and can produce and deliver therapeutic peptides to the brain.[29,30] Encapsulated cells have already been used for the therapy of diabetes mellitus,[31] amyotropic lateral sclerosis,[32,33] chronic pain,[34] Huntington's disease,[35] and for the treatment of malignant brain tumors.[36-38]

Step 2: Preclinical studies

Our group conducted a preclinical study testing the effect of encapsulated native MSCs and encapsulated glucagon-like peptide-1 (GLP-1) transfected MSCs in experimental traumatic brain injury (controlled cortical impact—CCI).[39]

GLP-1 is an endogenous insulin-stimulating peptide that is secreted from the gastrointestinal tract in response to food intake.[40] GLP-1 receptors are also expressed throughout the mammalian brain.[41] Stimulation of these receptors is associated with neuroprotective and neurotrophic activity.[42-44] GLP-1 has been shown to improve learning and memory in GLP-1 receptor-deficient mice.[45] The blood-to-brain delivery of native GLP-1 is, however, affected because GLP-1 rapidly degrades, with a plasma half-life of between 1 and 2 min.[46] Hence, the cells were used as a "bioreactor" which constantly releases GLP-1, while simultaneously bypassing the blood-brain barrier.

A human bone marrow-derived, mesenchymal stem cell line was used in this study. This cell line was immortalized by transduction with the human telomerase reverse transcriptase (hTERT) gene.[47] Following transfection with a plasmid vector encoding a GLP-1 fusion gene, the cells produced 8.7 kDa of dimeric GLP-1. The cells were alginate encapsulated and stored in liquid nitrogen until used. Each capsule contained approximately 2300 cells. Animals were randomized into five groups: controls (no CCI); CCI-only; CCI + native human bone-marrow derived mesenchymal stem cells (hMSC); CCI + GLP-1 producing hMSC; and CCI + empty capsules. Twenty capsules were implanted into the right lateral ventricle immediately before CCI. Even though this technique does not mimic the clinical setting, it was necessary in order to ensure implantation of the encapsulated cells into the ventricle, since the standard stereotactic coordinates become invalid after the CCI due to contusion-

related brain tissue shifting. On day 14, cisternal cerebrospinal fluid (CSF) was sampled for measurement of GLP-1 concentration, and brains were immuno-histochemically assessed using specific antibody staining for NeuN, MAP-2 and glial fibrillary acidic protein (GFAP). In order to determine the viability and the GLP production of the GLP-1 secreting hMSCs, nine healthy animals were implanted with 20 capsules using the same stereotactic technique. Capsules were retrieved, and viability and GLP-1 production rate was assessed after 2, 7, and 14 days of cerebral transplantation. One third of the retrieved capsules were stained with propidium iodide (staining of nonvital cells) and SYBR Green (staining of vital cells), and then visualized using fluorescence microscopy. The remaining capsules were recultured to measure the GLP-1 production rate.

In both of the stem cell-treated CCI groups, hippocampal cell loss was reduced, along with an attenuation of cortical neuronal and glial abnormalities, as measured by MAP-2 and GFAP expression. Anti-NeuN staining demonstrated a major reduction of positively stained neurons in the hilus of the dentate gyrus in the CCI-only and CCI with empty capsule groups. This neuronal loss was not observed in CCI animals implanted with native hMSCs and with GLP-1-producing hMSCs. Similarly, both Anti-GFAP and Anti-MAP-2 staining illustrated that the staining pattern in the animals with native and GLP-1 producing stem cells were very similar to those of the healthy controls, whereas in the CCI-only and CCI with empty capsules groups, increased immunostaining was observed, indicating reactive neuronal and glial changes. However, the effects were more pronounced in animals treated with GLP-1 secreting hMSCs. In the CCI animals with GLP-1 producing hMSCs, the CSF concentration of GLP-1 at day 14 was 17.3±3.4 pM. This concentration was significantly higher than that in the remaining groups: 3.1±1.6 pM (CCI + capsules without cells), 3.3±2.9pM (CCI + native hMSC) and 2.4±0.7pM (CCI-only). No measurable GLP-1 concentrations (detection limit: 2 pM) were found in the healthy control group.

Following a temporary cerebral implantation in healthy rats, the mean in vitro GLP-1 production rate of the hMCS explanted at day 2 was 3.68±0.49 fmol/capsule/h. On day 7 the rate was 2.85±0.45 fmol/capsule/h, and on day 14 it was 3.53±0.55 fmol/capsule/h. The production rate of non-implanted capsules was 7.03 fmol/capsule/h. Thus, the in vitro production rate of the encapsulated GLP-1 stem cells, retrieved after temporary implantation in healthy rats, was maintained at about half the rate of the nonimplanted GLP-1 secreting stem cells. Independently of the duration of implantation, propidium iodide and SYBR green fluorescence microscopy revealed that more than 95% of the stem cells were viable in the explanted capsules.

In a second study,[48] we tested the encapsulated cells described above in a double transgenic mouse model of Alzheimer's disease (AD) after intraventricular implantation at 3 months of age. Mice carrying mutations in the amyloid precursor protein and presenilin-1 and -2 genes develop AD-like deposits composed of A-beta at an early age. Since A-beta deposits as well as inflammation of the CNS are visible at 3 months starting in the frontal cortex, stem cell implantation was performed at this age to test whether early treatment may prevent the onset of A-beta deposition and associated inflammation. A-beta 40/42 deposition, and glial (GFAP) and microglial (CD11b) immunoreactivity were investigated 2 months after transplantation of either native MSC or MSC transfected with GLP-1 and compared with untreated controls. CD11b immunostaining in the frontal lobes was significantly decreased in the GLP-1 hMSC group compared with the untreated controls. Also, the plaque-associated GFAP immunoreactivity was only observed in one animal in the GLP-1 MSC group. A-beta 40 whole brain (enzyme-linked immunosorbent assay, ELISA) was decreased in both hMSC groups: 86.06 +/- 5.2 pg/mL (untreated control) vs 78.67 +/- 11.2 pg/mL (GLP-1 MSC group) vs 70.9 +/- 11.1 pg/mL.

According to these experimental findings, encapsulated native hMSCs possess anti-inflammatory and neuroprotective properties, which seem to be enhanced by genetical engineering of the cells to secrete GLP-1. Therefore, GLP-1-secreting hMSC capsules may have a therapeutic potential in acute but also chronic neurological diseases.

Step 3: Clinical translation of encapsulated mesenchymal cell biodelivery of GLP-1

Translating our experimental findings, intracerebal hemorrhage (ICH) was chosen as disease model to investigate the safety of encapsulated mesenchymal cell biodelivery of GLP-1 in a phase I/II trial which is currently ongoing.[49] Microencapsulated allogenic hMSCs are transplanted into the brain tissue cavity after neurosur-

gical evacuation of the hematoma. The objective of this approach is to improve the outcome after surgery for ICH; the local, neuroprotective, and anti-inflammatory cell therapy is targeting the secondary neuronal injury in the perihematomal area occuring in the first weeks after the bleeding.

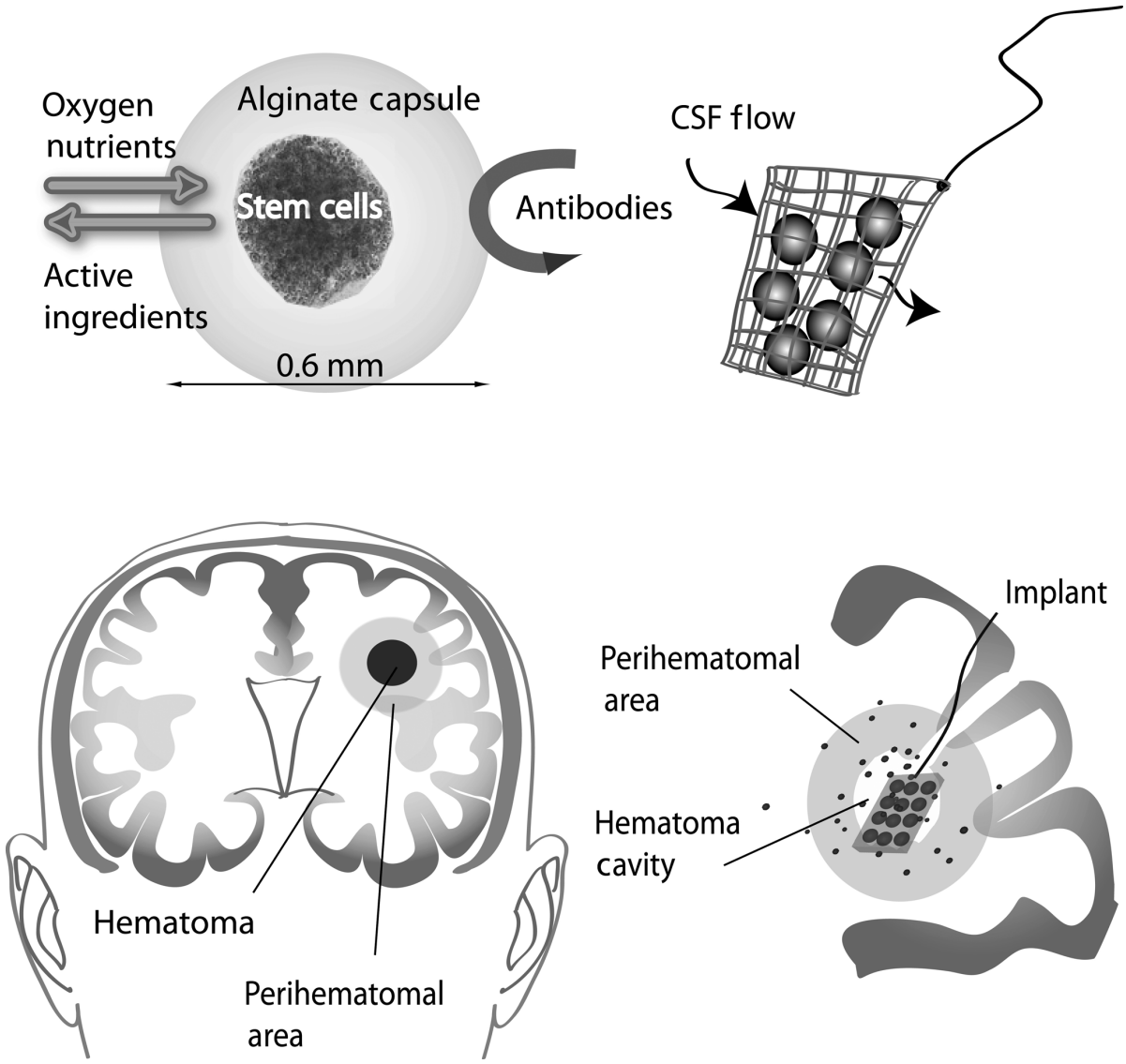

Figure 1. Encapsulated mesenchymal cell biodelivery of GLP-1. *Upper left:* Human bone marrow-derived, mesenchymal stem cells producing GLP-1 are encapsulated with alginate (capsule diameter 500 to 600 µm, each capsule containing 3200 cells). As the capsules permit the free passage of nutrients, oxygen, and, indeed, smaller molecules, the cells are maintained within the capsules, and can produce and deliver therapeutic peptides to the brain. At the same time, cells transplanted into the brain are protected from the immunological graft-versus-host response. *Upper right:* The microcapsules are filled into a 1.5 x 1.5 cm-sized bag that is manually sutured from a polypropylene mesh with pores of up to 300 µm. A 5-cm tether for fixation of the implant to the skull surface is applied. CSF can pass through the pores providing the encapsulated cells with nutrients and oxygen. *Lower left:* The surgical hematoma is evacuated leaving the perihematomal area. *Lower right:* The mesh bag is implanted into the hematoma cavity, and it is removed 2 weeks after implantation by a second surgery. GLP, glucagon-like peptide; CSF, cerebrospinal fluid.

In the clinical trial, each microcapsule contains about 3000 GLP-1 hMSC capsules, and approximately 7.8 x 106 cells are implanted. Since approval agencies are concerned about possible long-term side effects due to stem cell transplantation, the cells are not implanted into the brain directly, but filled into a 1.5 x 1.5 cm-sized bag that is manually sutured from a polypropylene mesh with pores of up to 300 µm. A 5-cm tether for fixation of the implant to the skull surface is applied. After surgical hematoma evacuation, this mesh bag is implanted into the hematoma cavity, and it is removed 2 weeks after implantation by a second surgery. *Figure 1* illustrates the delivery system.

Safety assessments include MRI examinations, physical and neurological examinations, NIH stroke scale (NIHSS), Barthel Index (BI), clinical laboratory profile, and any adverse events. Different risk levels are defined that would eventually lead to the explantation of the containment including the study medication (eg, systemic infection, local inflammatory reaction, anaphylactic reaction, seizures, unexpected neurological deterioration or other unexpected adverse events). Follow-up examinations continue until 6 months after surgery.

The interim evaluation of the first 11 patients revealed neither side effects from the surgical interventions nor implant-related side effects. Also, up to 30% of the transplanted MSCs survived the 2-week implantation period and were still secretorily active after explantation. The trial is still recruiting; a thorough assessment of the application safety of the novel therapy, including a comprehensive analysis of neurological, radiological, and laboratory parameters will be possible after completion of the trial including a total of 20 cases.

Step 4: Encapsulated cell biodelivery in TBI

According to the existing preclinical studies and the preliminary results of the ongoing clinical trial in ICH patients, GLP-1-secreting hMSC capsules might be an effective treatment for TBI patients as well. Presumably, the neuroprotective and anti-inflammatory properties of the cell capsules are most effective in the acute stage after TBI preventing ongoing secondary brain injury. However, additional preclinical studies are required to ascertain that the transplantation of cell capsules does not increase the risk of edema or may cause increased ICP. However, the preliminary radiological (MRI) results in the ICH patients suggest that the cell capsules may even decrease cerebral edema.

Additionally, preclinical work must address the application technique. Currently the therapeutic value of intracerebral injection of cell capsules into a traumatic lesion, ie, cerebral contusion, or into the cerebral ventricles is not established. The intraventricular application has been shown to be effective in our rodent TBI model; however, it is controversial as to whether this application route is also effective in humans. While the cerebroventricular administration of trophic factors has influenced the pathology of neurodegenerative disorders,[50,51] the rapid clearance of CSF into the venous circulation has been recognized as a substantial limitation to the pharmakokinetics of this drug delivery route.[52,53] The only reported clinical study investigating intraventricular, hollow fiber encapsulated cell biodelivery revealed only minimally increased CSF concentration of the delivered factor.[54] However, microencapsulation, as used in our clinical study, allows for the transplantation of a significantly higher number of cells, ie, millions compared with only hundreds of thousands in the hollow fiber encapsulation.[55-57] Thus, the higher release rates that can be achieved with the microencapsulation technique might compensate for the rapid CSF clearance, and thereby build up pharmacologically active CSF factor concentrations.

Also, it is not clarified, whether an enclosure, similar to the mesh bag used in the ICH trial, is necessary for intraventricular or intracerebral implantation. It might be safe and effective to inject the cell capsules without such containment. However, to validate this application, additional preclinical work addressing mainly acute and chronic safety issues is required.

Outlook

While encapsulated cell biodelivery has a reasonable perspective for a clinical application in traumatic brain injury, the translation of the existing findings requires extensive additional experimental studies. ❑

Traducción a la clínica de la terapia con células madre en el daño cerebral traumático: el potencial biogenerador de células mesenquimáticas encapsuladas del péptido-1 tipo glucagón

El daño cerebral traumático sigue siendo una importante causa de muerte e incapacidad, y se estima que anualmente afecta a diez millones de personas. Los estudios preclínicos han mostrado el potencial valor terapéutico de los tratamientos con células madre. Mediante los estudios preclínicos se ha sugerido que el mecanismo de acción depende de las propiedades neuroprotectoras y regeneradoras de las células madre. Sin embargo, hasta la fecha la terapia con células madre ha sido poco estudiada en ensayos clínicos. Este artículo resume la evidencia experimental actual y menciona los obstáculos para la aplicación clínica. Enfocándose en la terapéutica celular durante la etapa aguda del daño cerebral también se discute el potencial biogenerador de células encapsuladas como una nueva aproximación en la terapia celular.

Translation clinique du traitement par cellules souches des lésions cérébrales traumatiques : le potentiel de biodélivrance des cellules mésenchymateuses encapsulées du peptide-1 analogue au glucagon

Les lésions cérébrales traumatiques restent une cause majeure de décès et de handicap ; on estime que 10 millions de personnes par an sont touchées. Des études précliniques ont montré la valeur thérapeutique potentielle des traitements par cellules souches. Les propriétés neuroprotectives comme régénératives des cellules souches semblent être le mécanisme d'action retrouvé dans les études précliniques. Cependant, jusqu'à maintenant, le traitement par cellules souches n'a pas été largement étudié dans les études cliniques. En se concentrant sur le traitement au stade aigu des lésions cérébrales, nous analyserons le potentiel de la biodélivrance des cellules encapsulées comme nouvelle approche de thérapie cellulaire.

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